Investigation of the Transformation of Uranium under Fe^{III}-Reducing Conditions: Reduction of U^{VI} by Biogenic Fe^{II} in Green Rust

Edward O'Loughlin[1], Kenneth Kemner[1], Shelly Kelly[1], Maxim Boyanov[1], Bruce Ravel[1], and Russell Cook[2]

[1]Biosciences Division and [2]Electron Microscopy Center, Argonne National Laboratory, A U. S. Department of Energy Laboratory Managed by the University of Chicago

Michelle Scherer[1], Martin St. Clair[2], Sharon L. Smith[1], and Justine O. Harrison[1]
[1]Department of Civil and Environmental Engineering, University of Iowa and [2]Coe College, Department of Chemistry

Biogenic Green Rust Formation Resulting from Dissimilatory Iron Reduction

Defined mineral medium (Ona-Nguema et al., 2002 *ES&T* 36(1):16-20) containing 75 mM e- donor and 80 mM Fe(III) in sealed serum bottles was sparged with Ar and inoculated at an initial cell density of 5 X 10⁹ cell mL⁻¹. The suspensions were incubated at 30 °C in the dark. The reduction of Fe(III) was monitored by using the ferrozine assay to measure the Fe(II) content of 0.5 M HCl extracts of the suspensions. Biomineralization products were identified by X-ray diffraction (XRD), Mössbauer spectroscopy, and scanning electron microscopy (SEM).

### Reduction of Lepidocrocite by *Shewanella* spp.

96 d
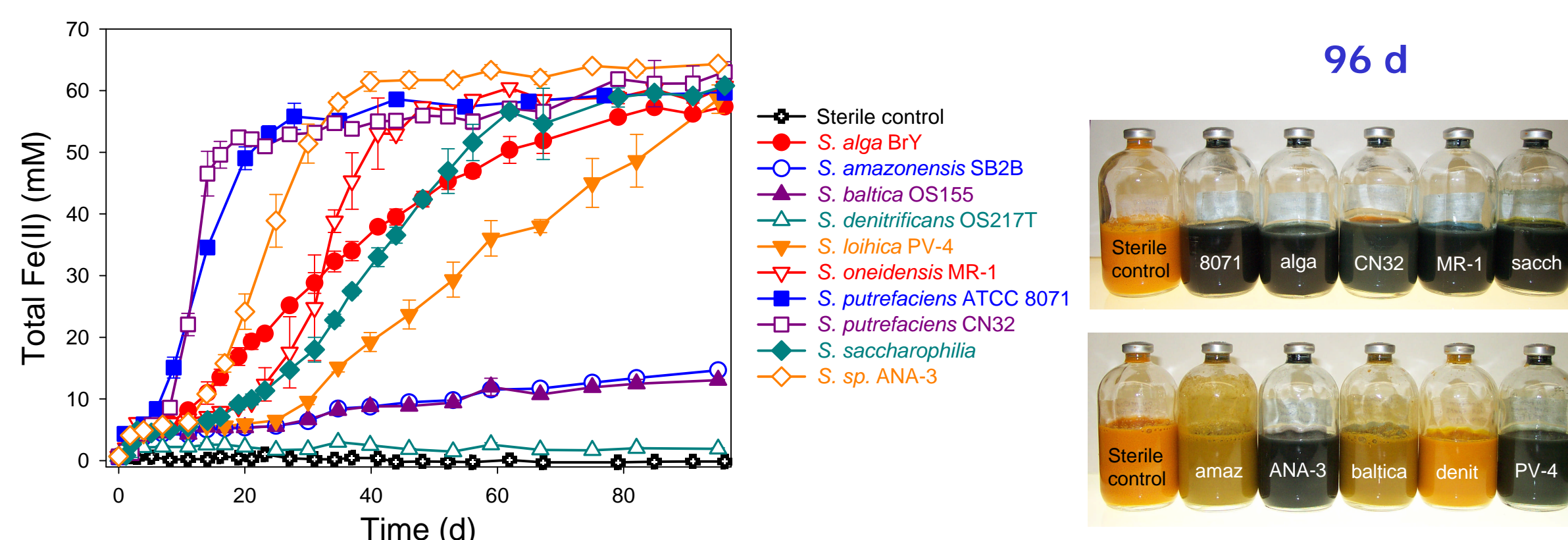
Lepidocrocite Reduction by *S. putrefaciens* CN32

Mossbauer Spectroscopy

X-Ray Diffraction
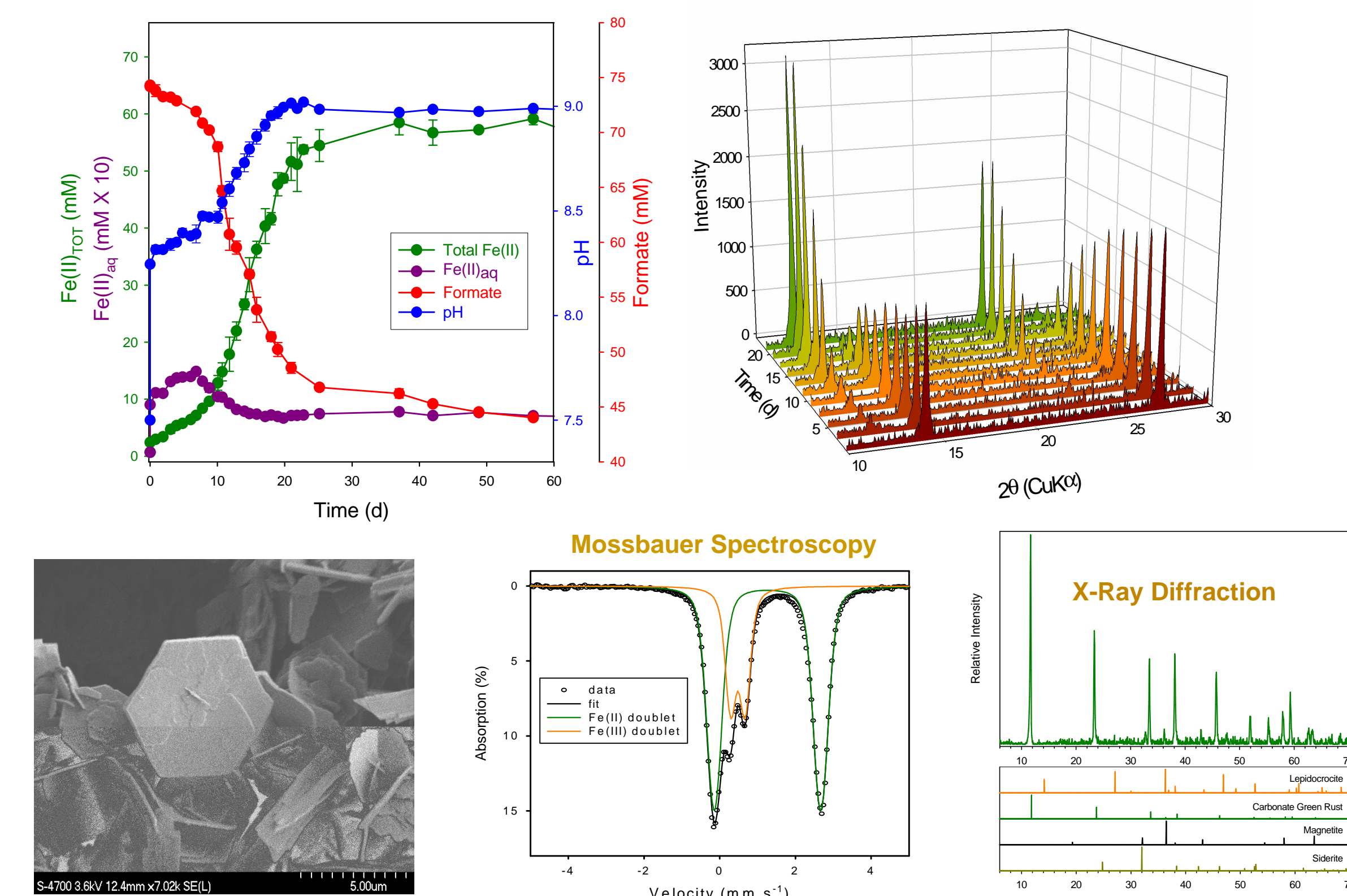
Analysis of the solids remaining after 57 d by SEM, Mössbauer spectroscopy and XRD indicated that the lepidocrocite was completely transformed by *S. putrefaciens* CN32, with carbonate green rust as the only significant solid-phase Fe(II)-bearing product. Carbonate green rust was also the only Fe(II) solid phase formed from lepidocrocite reduction by the other *Shewanella* spp. examined (with the exception of *S. denitrificans* OS217T).

e⁻ Donor Effects on Reduction of Lepidocrocite by *S. putrefaciens* CN32
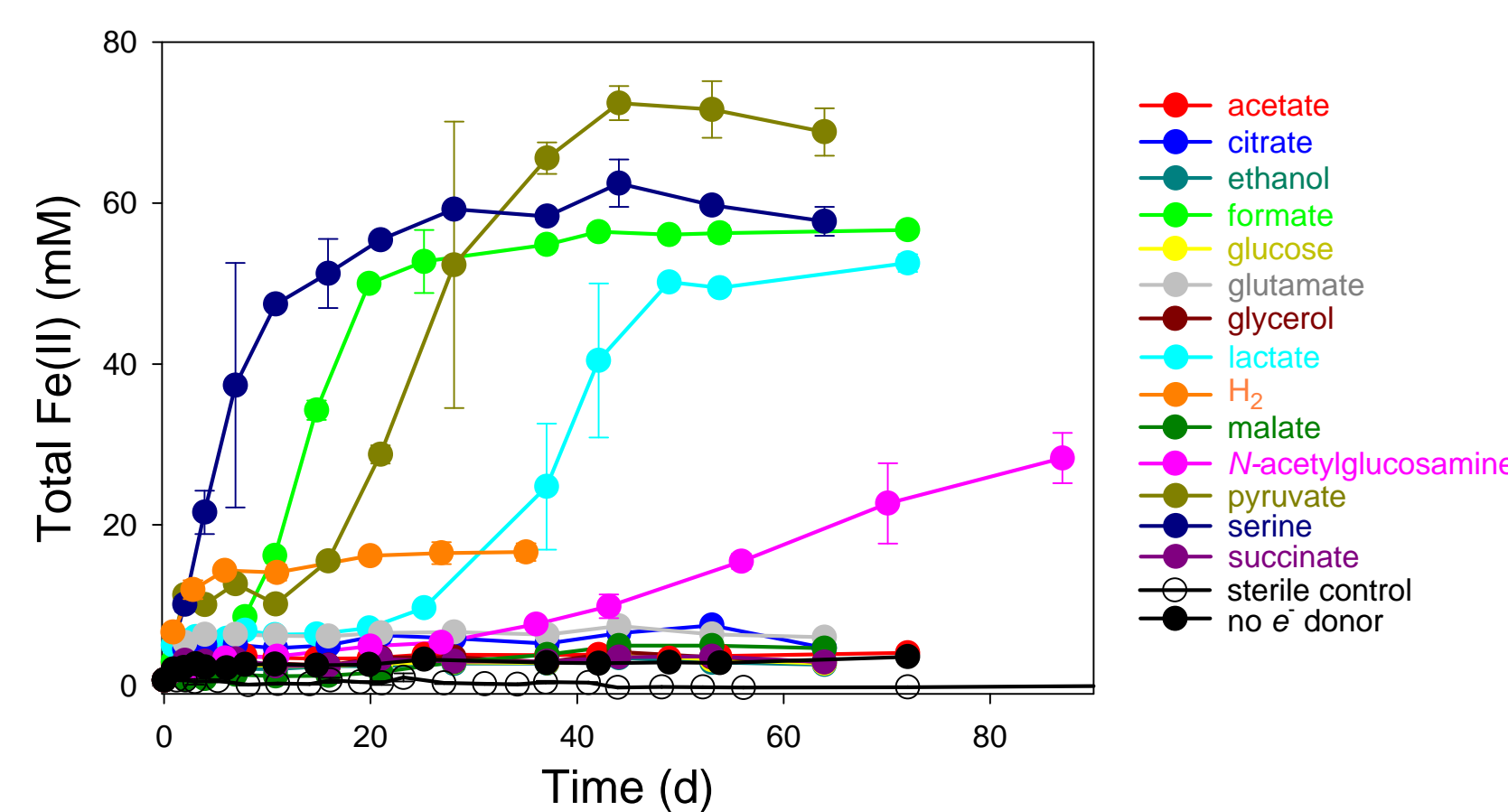
Of the e- donors tested, formate, H₂, lactate, *N*-acetylglucosamine, pyruvate, and serine supported significant Fe(III) reduction. Fe(II) production in the absence of added e- donor is attributed to utilization of endogenous e- donor(s)

e⁻ Donor Effect on Solid-Phase Fe(II) Products

Siderite (FeCO₃) was the dominant Fe(II) biomineralization product when serine or pyruvate were provided, while green rust was the dominant product with formate, H₂, lactate, and *N*-acetylglucosamine.
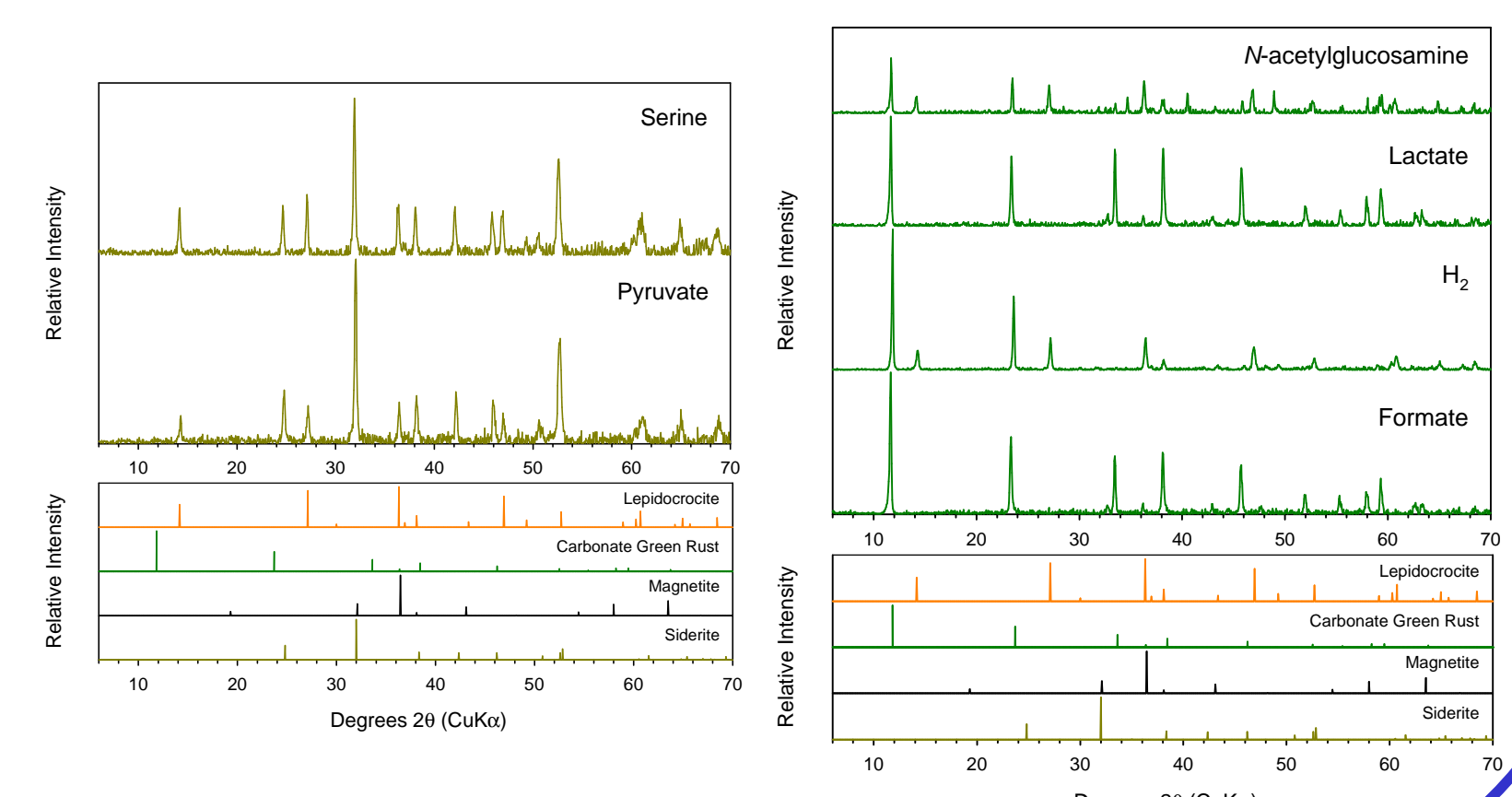
Reduction of U(VI) by Biogenic Green Rust

Preparation of Biogenic Green Rust

Biogenic green rusts were prepared by the reduction of lepidocrocite by *S. putrefaciens* CN32, *S. alga* BrY, and *S.* sp. ANA-3 as described in the panel to the left. The biogenic green rust suspensions (containing ~60 mM Fe(II)) were pasteurized at 70 °C for 1 h; this treatment was sufficient to kill the *Shewanella* spp., with minimal alteration of the biogenic green rust. The pasteurized biogenic green rust was then repeatedly sonicated and washed with DI water. The initial pH of these suspensions ranged from 7.3 – 7.5.

U(VI) Interaction with Biogenic Green Rusts

The biogenic green rust suspensions were spiked with a stock solution of uranyl chloride. The resulting suspensions had initial U(VI) and Fe(II) concentrations of 625 µM and 60 mM, respectively. After 48 h, the pH of the suspensions was measured; the suspensions were centrifuged, and the supernatants were saved for uranium analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Uranyl was readily removed from solution in the presence of biogenic GR. Within 48 h, solution-phase uranium concentrations decreased from 625 µM to 1.6 µM. The final pH of the suspensions ranged from 5.8 – 6.0.

EXAFS Analysis of U associated with Biogenic Green Rust

The solids remaining after centrifugation (hereafter designated as UbioGR) were analyzed by extended X-ray absorption fine structure (EXAFS) spectroscopy. Measurements at the U L3 absorption edge (17166 eV) were performed at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID at the Advanced Photon Source (APS) at Argonne National Laboratory. Energy scans were collected at different locations on the sample to reduce radiation exposure; however, no time-dependent change in the data was observed for any of the samples.

The EXAFS data were fit with a model based on the structure of uraninite (UO₂) consisting of 8 oxygen atoms at 2.35 Å (O1), 12 uranium atoms at 3.87 Å (U1), and 24 oxygen atoms at 4.49 Å (O3), but modified to include contributions from 1-3 Fe atoms and a splitting of the O1 shell (O1a and O1b); see table below.

Neighboring atom	Microbe Type	Number of neighbors	Distance to Neighbors (?)	Disorder in distance to neighbors (X10⁻³ ?²)
U-O1a	CN32	6.8 ± 0.4	2.31 ± 0.01	13.9 ± 1.7
	ANA3	7.2 ± 0.5		
	Alga	6.8 ± 0.4		
U-O1b	CN32	1.2 ± 0.4	2.67 ± 0.05	13.9 ± 1.7
	ANA3	0.8 ± 0.5		
	Alga	1.2 ± 0.4		
U-Fe	CN32	1.9 ± 0.9	3.41 ± 0.02	10.4 ± 5.0
	ANA3	1.8 ± 0.7		
	Alga	2.0 ± 0.8		
U-U	CN32	3.3 ± 1.3	3.77 ± 0.03	10.4 ± 5.0
	ANA3	4.8 ± 1.4		
	Alga	3.8 ± 1.1		
U-O2	CN32	6.6 ± 2.5	4.43 ± 0.03	20.8 ± 10.0
	ANA3	9.6 ± 2.8		
	Alga	7.7 ± 2.2		

Fit Results

Fit results for the EXAFS data obtained from UBioGR samples. The average number of Fe atoms is consistent with 1 – 3 Fe neighbors per U atom. The U(IV) appears to be present as small uraninite clusters containing 2 – 6 U atoms on average.
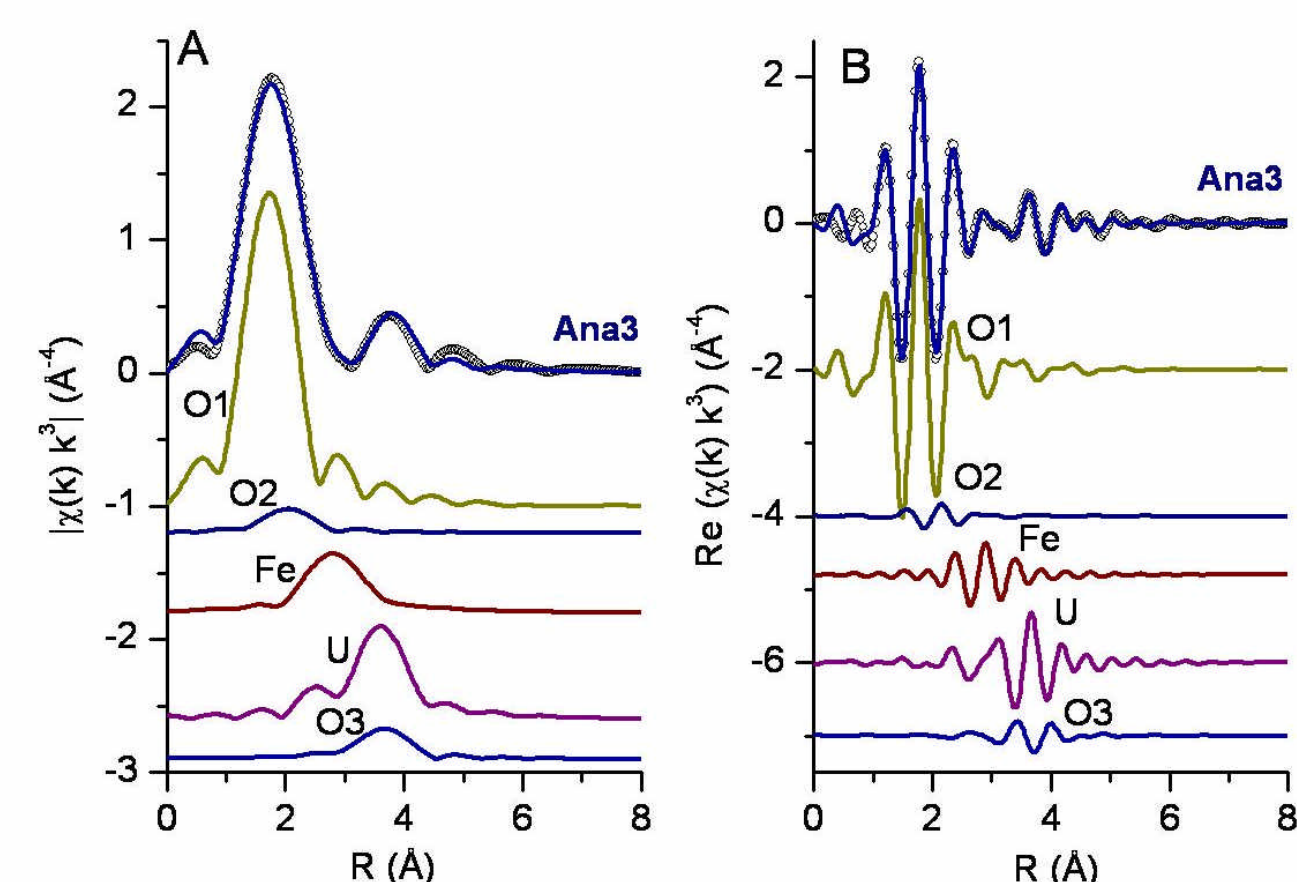
EXAFS Data

EXAFS data (symbols) of UBioGR samples with the corresponding model fits indicated by the solid lines. The fit range was 3.5 – 7.5 Å⁻¹.
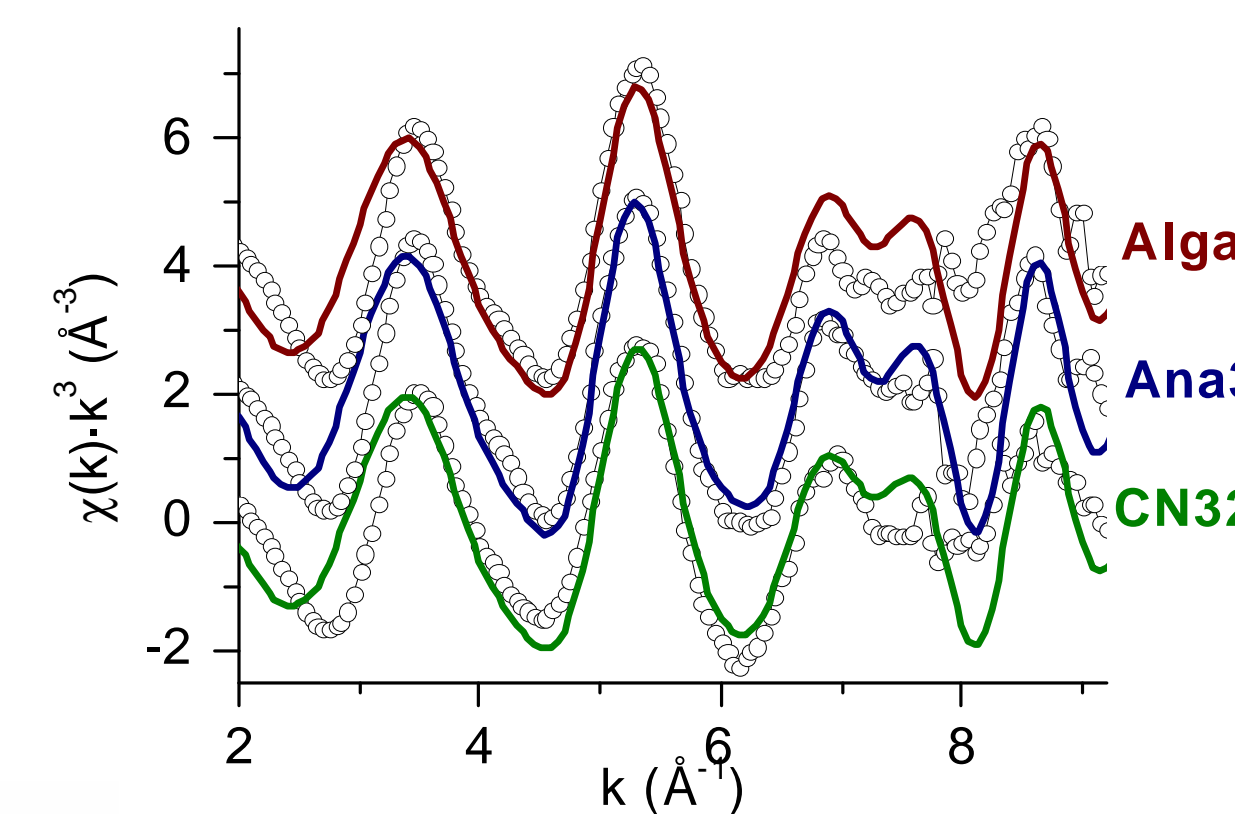

Model Components

The magnitude (A) and real part (B) of the Fourier transform of the EXAFS data (symbols) from the *S.* sp. ANA-3 UBioGR sample and the model fit (top blue curve). The components of the model corresponding to the various coordinating atoms are shown by the curves below the EXAFS data.


Fourier Transforms of UBioGR Data

The magnitude (A) and real part (B) of the Fourier transform of the EXAFS data (symbols) from the *S. alga* BrY, *S.* sp. ANA-3, and *S. putrefaciens* CN32 UBioGR samples and the model fit (lines).
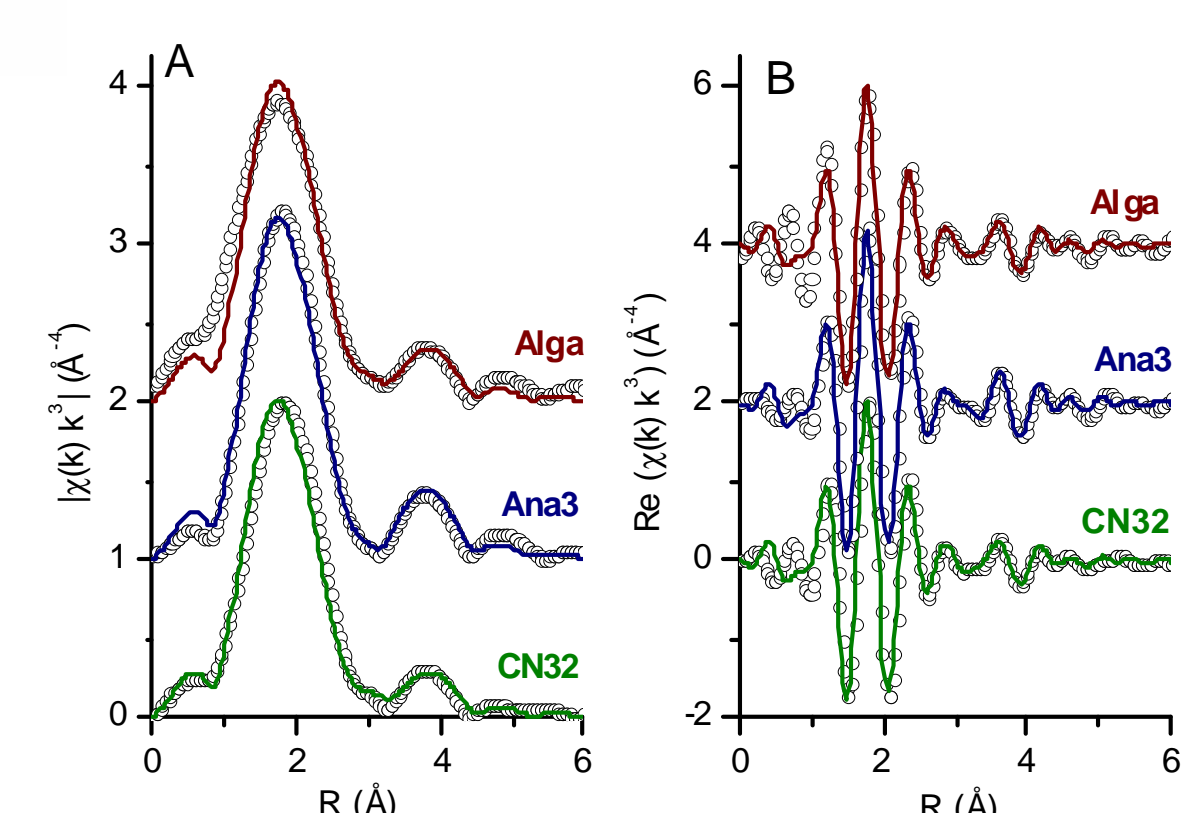
The results of the EXAFS data analysis indicate that U(VI) is reduced to U(IV) by biogenic green rusts produced by several species of *Shewanella*. In these systems the U(IV) is present as small molecular clusters with a structure similar to uraninite. These clusters appear to be associated with Fe.

Interlayer Anion Effects on the Reduction of U(VI) by Green Rust

Hydroxide Layer
Hydrated Interlayer
Hydroxide Layer
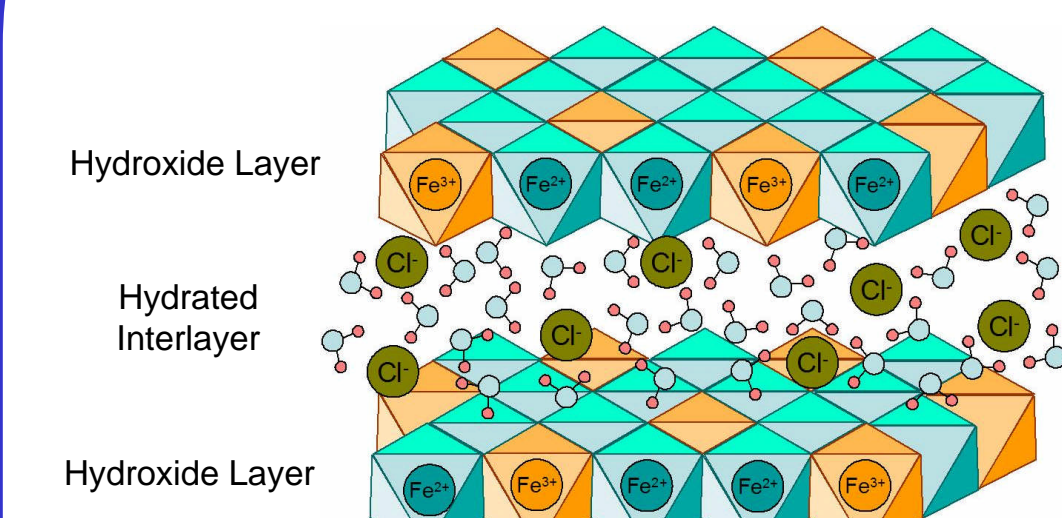
The structure of green rusts consists of alternating positively charged hydroxide layers and hydrated anion layers. Substitution of Fe³⁺ for Fe²⁺ in the Fe(OH)₂ sheets confers a positive charge to the hydroxide layer that is balanced by hydrated anions in the interlayer (typically Cl⁻, SO₄²⁻, or CO₃²⁻).

Green Rust Synthesis

Green rusts were synthesized by precipitation of a ferrous/ferric iron mixture, with appropriate anions, by the addition of NaOH (sulfate, chloride) or Na₂CO₃ (carbonate) to pH 8. The green rust precipitate was filtered and freeze dried under anoxic conditions to produce a fine blue-green powder that was confirmed to be green rust by powder x-ray diffraction.

Kinetics of U(VI) Removal

Kinetic data were collected from batch reactors containing sulfate, chloride, or carbonate green rust at a solids loading of 1 g L⁻¹ and a solution of 400 µM U(VI) in 0.1 M TAPS buffer at pH 8.0. Samples were collected throughout the experiment and passed through a 0.2 µm filter. Aqueous U(VI) concentrations were measured by ion chromatography (DeBeer and Coetze, 1992, *Radiochim. Acta.* 57:113-117).
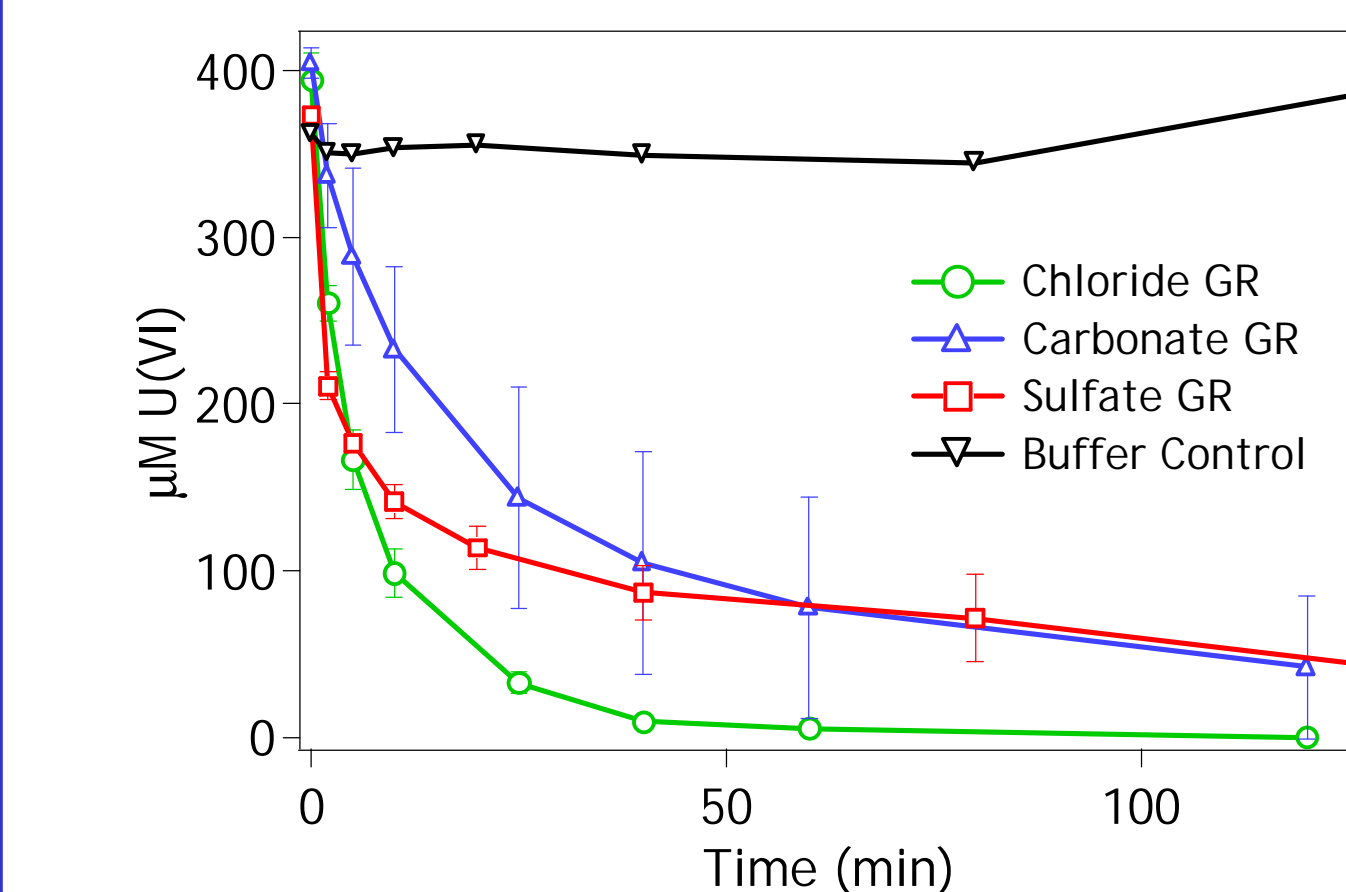

Removal of U(VI) from solution

Although there are differences in the extent of uptake, > 90% of the removal of U(VI) from solution occurred within 50 min for each of the green rusts examined..

U(VI) Adsorption or Reduction?

To determine the whether U(VI) is adsorbed or reduced by the green rusts, XAFS spectroscopy was used to distinguish U(VI) from U(IV) in the product as well as provide information about the coordination environment of the uranium atom.
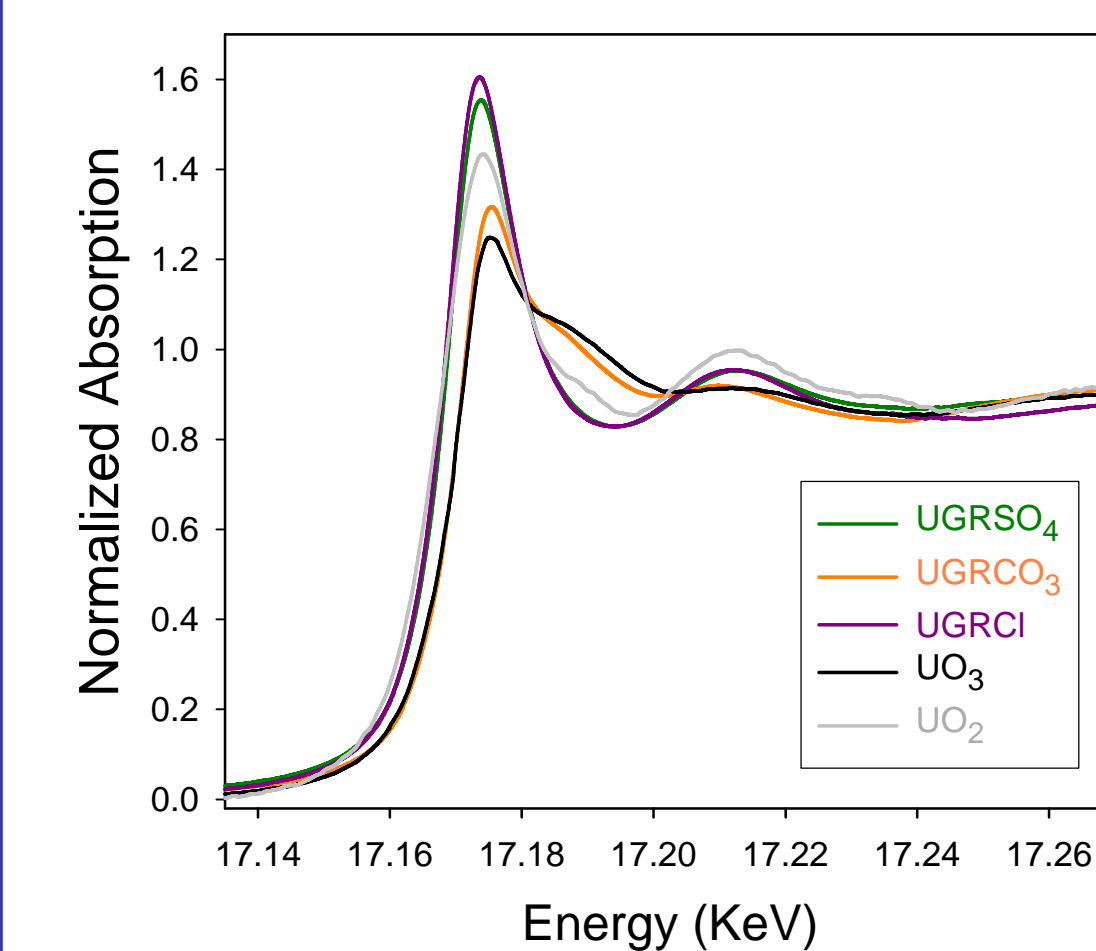

XANES spectra for UGRCO₃, UGRCl, and UGRSO₄ and for U(IV) and U(VI) standards

A comparison between the U–XANES spectra of the U(IV) and U(VI) standards and UGRCO₃, UGRCl, and UGRSO₄, clearly shows that the U(VI) added to GRCl and GRSO₄ suspensions is reduced to U(IV). Conversely, there was no indication of U(VI) reduction to U(IV) in GRCO₃ suspensions.

EXAFS results for UGRCO₃, UGRCl, and UGRSO₄.

The peak representing the U-U peak in the Fourier transformed EXAFS data for UO₂ is highly attenuated in the data for GRCl and GRSO₄, which is consistent with molecular clusters of UO₂. Peaks corresponding to the equatorial and axial oxygen atoms in uranyl are evident in the data for GRCO₃.
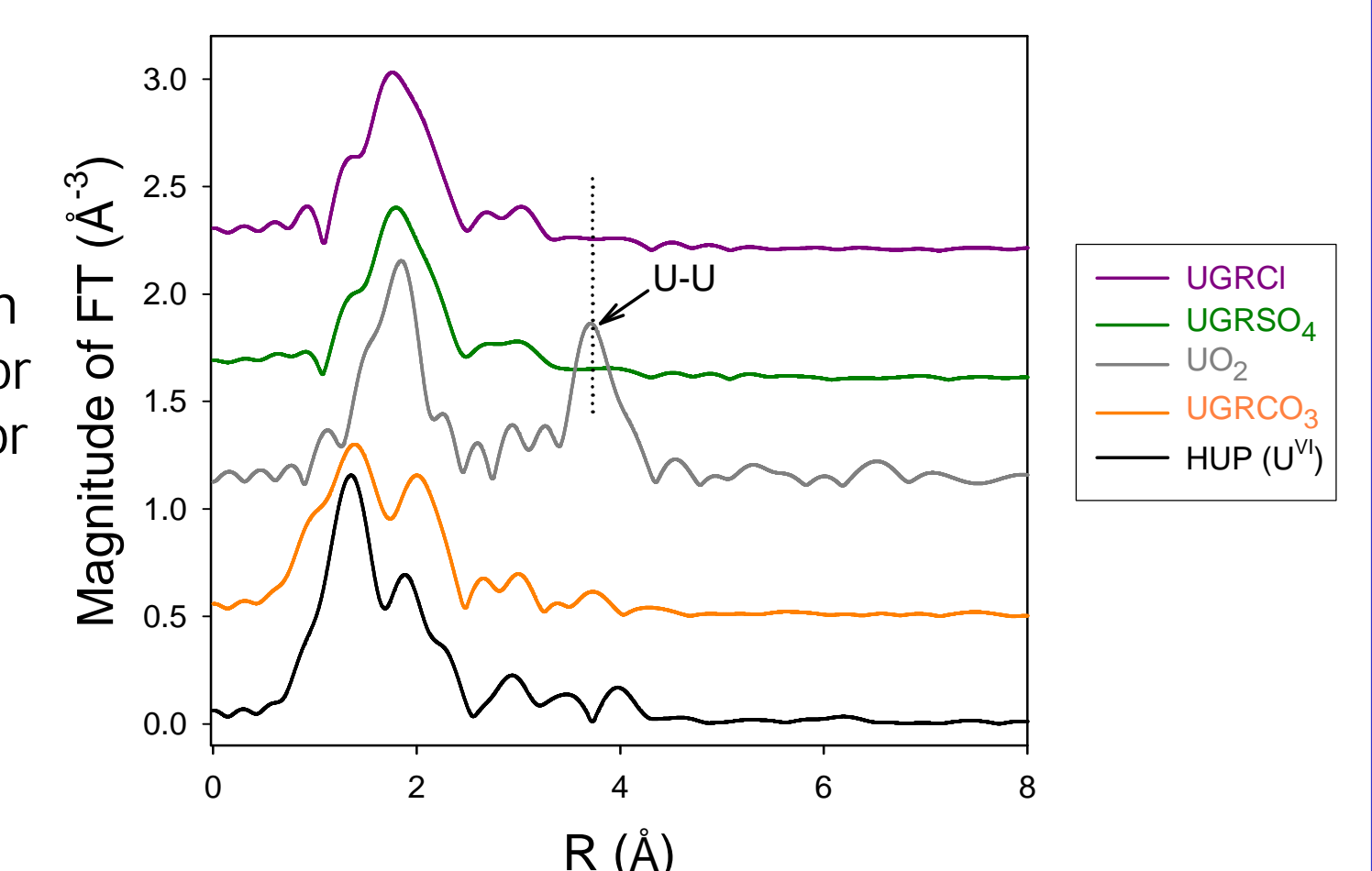
In GRCl and GRSO₄ suspensions > 99% of added U(VI) is removed from solution with > 90% reduced to U(IV). The EXAFS data for the resulting U(IV) phase are consistent with the formation of single molecules or small molecular clusters of UO₂. Although > 80% of U added to the GRCO₃ suspensions is associated with the solid phase, there is no indication of significant (> 10%) reduction to U(IV). These results clearly indicate that the reactivity of green rusts with respect to the reduction of U(VI) to (UIV) is affected by the nature of the interlayer anion.